IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Application of: | Docket No. 32265 |
| STOUT, Robert L. | Customer No.: 23589 |
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| Filed: January 18, 2002 | Confirmation No.: 7968 |
| METHODS OF DETERMINING CHRONIC HEPATITIS C INFECTION | Examiner: HORNING, Michelle |

Mail Stop Amendments
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 C.F.R. §1.132**

I, Mark E. Magee, do state and declare as follows:

1. I am currently the Vice President of Laboratory Operations at Clinical Reference Laboratory, Inc. (CRL), the assignee of the above-referenced patent application, where I have been employed since May 1978. In addition to my current position, I have held supervisory, research, assistant director, and technical positions at CRL over the past 30 years. I hold the following degrees: Bachelor of Science, Masters in Physiology, and Masters in Business Administration. I have experience in research and development, as well as laboratory management associated with Clinical Trials, Toxicology, and all phases of the clinical laboratory testing performed at CRL.

2. I understand that the Examiner has rejected the claims as being obvious in view of two references, Fabrizi et al. (hereinafter "Fabrizi"), and Fong et al. (hereinafter "Fong"). I have reviewed these references along with the instant application and have compared the claimed

invention to the disclosures of the cited prior art. I have been asked to provide this declaration to attest to what these references teach to a person skilled in the art, and the nonobviousness of the claimed invention in view of these references.

3. To be effective, antibody-based assays must be highly specific and have a high degree of sensitivity. Sensitivity is particularly important for detecting HCV infection because HCV is a hypermutable virus (i.e., has a high rate of spontaneous mutation). Thus, an assay using only a single antigen for detecting the presence of HCV antibodies may fail to detect a large number of individuals who are in fact anti-HCV positive.

Antibody-based assays must also have good positive and negative predictive values. Positive predictive value is the probability that an individual testing positive is in fact infectious (i.e., true positives). Negative predictive value is the probability that an individual testing negative is in fact non-infectious (i.e., true negatives). Thus, a test with good positive and negative predictive values will accurately identify true positives and true negatives with a high degree of probability.

4. The present invention provides a method for determining the probability of whether an individual has chronic HCV infection or has cleared the infection using antibody-based assays. At the time of the present invention, antibody tests were used primarily to document HCV exposure, but could not distinguish between infectious individuals (i.e., chronic) and non-infectious individuals (i.e., cleared) based upon positive assay results. Positive results indicated exposure only, and PCR techniques had to be performed to determine whether viral particles were still present, indicating a chronic infection. At the time of the present invention, the cost to confirm a single case of HCV infection using PCR was around $120 per test. Even today, PCR testing is still cost prohibitive, at about $100 per test, especially when testing a large number of samples/individuals. In comparison,

the cost of an antibody-based assay is about $10-12 per test. Antibody-based assays are also highly automated compared to PCR, requiring minimal clinician time and oversight.

In practice, the claimed methods allow one having ordinary skill in the art to positively identify individuals with a very low probability of having a chronic infection who have a high probability of being HCV RNA negative. This permits resources to be focused on a limited number of individuals who are then tested using the more expensive PCR test to confirm the presence or absence of HCV RNA. Likewise, the claimed methods can be used to positively identify individuals with a high probability of having a chronic infection who do need further testing. That is, depending upon which group is the focus of the testing, the present methods can be adapted so that one having ordinary skill in the art can identify individuals who have a very high probability of not being chronically infected or individuals with a very high probability of being infected, or both.

Advantageously, the present methods have a high degree of specificity and sensitivity, as providing a plurality of different antigens in a single assay to detect multiple HCV antibodies. In addition, according to a preferred embodiment, the claimed method tests samples using at least two different assays with a plurality of different antigens, further increasing the specificity of the test. These methods also have good positive predictive value and negative predictive value.

5. After carefully reading the Fabrizi and Fong references, according to my experiences in clinical and laboratory testing and according to the state of the art, a person having ordinary skill in the art, at the time of the instant invention, would not have been able to deduce or derive the claimed methods for determining the probability that an individual has developed chronic HCV infection or has cleared the infection using OD readings and antibody-based assays based upon the teachings of Fabrizi and/or Fong. In particular, to a person having ordinary skilled in the art, neither reference teaches or suggests that antibody-based assays can be used to qualitatively determine the

probability that an individual is chronically infected, and neither reference provides OD readings which are correlated with certain probabilities of chronic or cleared infection. That is, when taken either individually or combined, nothing in these references could support or explain the features claimed in the above-referenced application, as the present invention yields surprising and unexpected results over the prior art, as explained in more detail below.

FABRIZI ET AL.

6. Fabrizi is concerned with determining the significance of the IgM class of antibodies in HCV infection in patients with end stage renal disease (ESRD), who have also tested positive for anti-HCV IgG activity in serum. At first glance, Fabrizi purports to disclose a correlation between the prevalence of anti-HCV IgM antibodies and the presence of HCV RNA in serum (in patients with ESRD) that would permit a determination of the probability that the individual has chronic HCV infection or has cleared the infection. However, the teachings of Fabrizi focus only on a one-way correlation and fail to examine the entirety of the data, emphasizing only the favorable statistics. That is, a closer look at the data as a whole reveals that the results are inconclusive at best. Although Fabrizi purports to demonstrate high specificity and a positive predictive value, the test had terrible sensitivity and poor negative predictive value. The results from Fabrizi have been presented in Table 1 below to aid in understanding the true significance of the data and facilitate the following discussion.

**Table 1**

| 78 total patients | 45 total RNA positive | | 33 total RNA negative |
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| **17 total Anti-HCV Positive** | 15 - true positives | | 2 - false positives |
| **61 total Anti-HCV Negative** | 30 - false negatives | | 31 - true negatives |

As shown in Table 1 above, in Fabrizi, testing positive for anti-HCV IgM, strongly suggests the presence of HCV RNA in serum, and thus infectiousness. Indeed, of the 17 patients who tested positive for anti-HCV IgM, 15 subsequently tested positive for HCV RNA (i.e., true positives). Thus, there were only 2 false positives, giving the test a positive predictive value. However, 45 patients of the 78 patients actually tested positive for HCV RNA in serum (Page 316, second full paragraph). Therefore, 30 individuals who tested negative for anti-HCV, actually tested positive for HCV RNA in serum. Thus, there were *30 false negatives*, giving the test a very poor negative predictive value. That is, **67% of the infectious patients were not identified by the antibody test.** Stated another way, the antibody test only identified one-third (15 out of 45) of the patients who were, in fact, chronically infected. Therefore, although testing positive for IgM was a strong indicator of chronic infection, testing negative for IgM did not mean the individual has cleared the infection. Thus, the teachings of Fabrizi would not permit one having ordinary skill in the art to differentiate between individuals having chronic infection and individuals who have cleared the infection.

7. Moreover, the lack of sensitivity demonstrated by Fabrizi's data would not be tolerated in a practical setting. That is, when surveying a set of samples for infection, missing two-

thirds of the infectious samples on the first pass (as in Fabrizi) would be unacceptable. As a practical matter, in order to be useful, the assay must allow a clinician to identify with a high degree of probability the *true negatives*, i.e., those individuals who *do not* need to undergo the expensive PCR test for verification. According to Fabrizi's data, although the presence of anti-HCV IgM antibody is a strong indicator of infectiousness, the high percentage of false negatives (67%) does not permit identification of those individuals who do not need to be tested using PCR. That is, the test only identifies those individuals who *do* need to undergo PCR testing. However, every member of the group must still be tested using the expensive PCR to determine whether there is HCV RNA in serum, because if an individual tests negative for anti-HCV IgM antibody, that does not mean they are not chronically infected. Thus, as a practical matter, the correlation disclosed in Fabrizi is not helpful, and does not provide the advantage of being able to eliminate non-infectious individuals on the first pass (i.e., during the first round of testing with the antibody-based assays). Indeed, in the third full paragraph on page 316, Fabrizi discloses the poor negative predictive value and sensitivity for the anti-HCV IgM assay, but fails to acknowledge or discuss these deficiencies.

8. When viewed in its entirety, the data in Fabrizi is not helpful and does not provide any meaningful disclosure that would lead one having ordinary skill in the art to use an antibody-based assay to distinguish between chronically infected and non-infectious individuals. Moreover, in the first full paragraph on page 317, Fabrizi acknowledges that data from this study is limited to ESRD patients. Fabrizi also acknowledges that while the significance of IgM antibody in other diseases appears to be clear, its significance in individuals with HCV is inconclusive, as evidenced by the number of conflicting reports cited on page 317.

9. Fong is concerned with the significance of certain antibodies to structural HCV antigens in HCV infection in patients with persistently normal liver tests. The study in Fong was comprised of thirteen patients with confirmed anti-HCV reactivity and normal liver tests over a period of 6 months. According to Fong, the samples were tested using "*individual* antigen ELISAs." (Page 254, third full paragraph) (emphasis added). That is, unlike the present invention, the samples in Fong were *not* contacted with a plurality of antigens. Rather, each individual antigen was "separately coated on to [*sic*] microwells." (Page 254, third full paragraph). The titers of antibodies reactive with the *individual* antigens were then observed and recorded. (Page 255, first full paragraph).

There is nothing in Fong that would suggest to one having ordinary skill in the art to use a plurality of antigens in a single assay. Rather, Fong actually teaches against this. That is, the *only* statistically significant correlation of antibodies to HCV infection status disclosed by Fong, is in regards to the anti-E2 titers. The remaining antigens, c22-3, c200, c33c, and c100-3 from ELISA, and c100-3, c33c, c22-3, and 5-1-1 from RIBA 2.0, were reported as being inconclusive. Based upon the teachings of Fong, one having ordinary skill in the art would have expected that including the other antigens in the assay would only obscure the results. Thus, based on the teachings of Fong, one having ordinary skill in the art, at the time of the present invention, would not have been motivated to include these other antigens in a single assay, or combine a plurality of antigens into a single assay, because they would have expected only E2 to yield legitimate results.

10. Even if one having ordinary skill in the art would be motivated to use a multi-antigen assay, one having ordinary skill in the art would have been discouraged from selecting the other antigens disclosed in Fong to use in this assay. That is, according to Fong, antigens c22-3, c200,

c33c, c100-3, and 5-1-1 do not provide an indication of the presence of HCV RNA in serum. Thus, one having ordinary skill in the art would have had to determine which other HCV antigens could be included in the assay to give an indication of the presence of HCV RNA in serum. However, Fong provides no guidance on selecting additional antigens beyond those antigens individually tested in the disclosed assays, and there is little predictability in this area. Selecting antigens to include in a multi-antigen assay would require that each sample be tested first with different antigens using the assay, and then with expensive and time-consuming PCR to confirm infection status. The results would then have been compared and analyzed to determine which antigens provided a statistically significant correlation to the presence of HCV RNA in serum. In addition, the tests would have to be performed on a sufficiently large and representative sample set. At the time of the present invention, this would have been quite expensive considering the costs for the assays and PCR tests for each sample, the machinery and equipment required to run these tests, the amount of clinician time, and the number of samples involved.

11. Fong specifically recognizes that "attempts to identify specific serologic markers which correlate with disease status in HCV infected patients have been largely unsuccessful." (Page 256, last paragraph). However, Fong also states that "ELISA testing using multiple HCV recombinant antigens demonstrated mean antibody titers to structural antigens, in particular E2 antibodies, to be significantly lower in nonviremic patients." (Page 256, fourth full paragraph). This statement is misleading, however, in that the *only* antibodies shown to have a statistically significant correlation were E2 antibodies. In addition, as explained above, although Fong performed ELISA testing using "multiple antigens," the reactivity to each antigen was *individually* tested. Thus, Fong did not actually run assays using multiple antigens in a single assay, as in the presently claimed methods.

Moreover, the fact that anti-E2 titers happened to be lower in nonviremic patients in Fong's study, does not necessarily permit this correlation to be applied to all HCV antigens or HCV infected individuals. As noted in Fong, E2 is a hypervariable sequence prone to rapid and spontaneous mutation. (Page 257, second full paragraph). One having ordinary skill in the art would recognize that although anti-E2 titer appeared to correlate with HCV RNA in serum in Fong's study, the hypervariability of this sequence makes detecting and monitoring the anti-E2 titer unreliable and prone to false negatives. It would also require that commercially-available assays be constantly updated in an attempt to keep up with the mutated sequences. Thus, as a practical matter, especially in a clinical setting, one having ordinary skill in the art would not use E2 antigens in an assay to determine the probability of whether the individual providing the sample has chronic HCV or has cleared the infection. Indeed, the working examples of the present application describe two commercially-available antibody-based assays that were used to perform the claimed method, neither of which contains E2 antigens or proteins encompassing the E2 antigen. Rather, the Abbott HCV EIA 2.0 assay contains c100-3, HC-31, and HC-34 antigens, and the ELISA ORTHO HCV 3.0 contains c22-3, c200, and NS5 antigens. As noted above, Fong actually teaches away from many of these antigens.

In addition, although Fong makes the correlation between the anti-E2 titers and HCV infection status, Fong does not provide any actual OD readings and does not correlate the test results with certain probabilities for being chronically infected or having cleared the infection.

12. Finally, the data from Fong has limited applicability, given the strict criteria for selecting eligible patients and the small sample set (13 patients total). That is, it is doubtful that any certain probabilities, if deduced from the data in Fong, would be applicable to general population modeling. Rather, to be useful, the sample needs to embrace all sources of variation that are

embraced by the target population (i.e., the population to which the results will be applied to make determinations on the probability of chronic vs. cleared HCV infection). For example, the working example in the present application tested 1,200 serum samples, where the only criteria was that the sample had an ALT concentration above 41 U/ml.

13. Based upon my experience in clinical and laboratory testing, one having ordinary skill in the art would not be motivated to combine the teachings of these two references. In particular, Fabrizi and Fong are each concerned with a very specific set of patients with defined symptoms and/or conditions. These patients are not representative of the population at-large, or even of the population of individuals testing positive for anti-HCV antibodies. Thus, one having ordinary skill in the art would recognize that attempting to extrapolate from these defined sample sets would not yield accurate results when the data is applied to the larger population. Moreover, because Fabrizi and Fong are each concerned with such defined groups of patients, one having ordinary skill in the art would not be motivated to combine data for individuals having ESRD who also tested positive for anti-IgG, with data for individuals testing positive for anti-HCV, who have persistently normal liver tests for 6 months, do not consume more than 80 grams of alcohol/day, do not have chronic liver disease, or HBsAg (hepatitis B surface antigen) or anti-HIV in serum.

14. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful, false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and such wilful false statements may jeopardize the validity of any patents issued from the patent application.

Date: 12 May 2008 Mark E Magee

Mark E. Magee